Remarks

This is in response to the Official Action mailed March 25, 2005.

Claims 1-21, 23 and 25-28 are pending. Claims 1-5, 12-14 17 and 12 have been amended. Claims 25-28 are new.

Reconsideration is requested.

Claim Rejections - 35 USC §112

Claim 3 has been rejected as not enabling for treatment of all types and forms of cancer. The rejection is traversed on the ground that claim 3 depends from claim 1 and so includes the tumor cell recitations found in claim 1. However, to advance prosecution the use of the word cancer has been replaced with "*a patient having a tumor that use the Hedgehog/Smoothened signaling for prevention of differentiation and/or for prevention of apoptosis of tumor cells*" (emphasis added). This amendment is seen to render the question moot. Withdrawal of this rejection is requested.

Claims 2 and 14 have been rejected as in definite for use of "transient administration" without antecedent basis. These claims have been amended to recite that the period of exposure *in vivo* to the medicament is one that causes a smaller proportion of or no normal tissue cells to undergo apoptosis and/or differentiation. The amendment is seen to remove the antecedent basis problem. Withdrawal of this rejection is likewise requested.

Claim Rejections - 35 USC§102 and 35 USC §103

Claims 1, 3-7, 10-11, 13-20, 23 and 24 have been rejected as being anticipated by Dudek et al. (understood to be US 6291516). Claims 2, 8-9, 12 and 21-22 have been rejected as obvious from Dudek et al. The rejections are traversed.

Method Claims 1-12 and 25-26.

The cited prior art document (*Dudek et al*) discloses a subject method and compositions which may be used for inhibition of proliferation of the cells displaying *Hedgehog/Smoothened* (*Hh/Smo*) signalling activity and for treatments of various conditions (referred generally as "proliferative disorders" in *Dudek et al*). The subject method referred to relates to the use of an unlimited number of compounds having the molecular framework of a steroidal alkaloid as well as other molecules. While a generic disclosure of an unlimitedly broad group of compounds is made in terms of structure, a relatively more defined disclosure is made with regard to the functional features of subject compounds. The subject compounds are said to bind to the *Patched* or *Smoothened* proteins to affect *Hh/Smo* signalling or to act to alter intracellular cAMP. Along with these compounds, *Dudek et al* disclose a large list of highly different therapeutic or cosmetic applications employing them. A very large number of pathological conditions (some known to have basic mechanisms of opposite nature) are said to be treatable.

The Applicants' invention and claims, on the other hand, are directed specifically and primarily to a method and compositions for inducing differentiation and apoptosis of the tumor cells that use the *Hh/Smo* signalling to inhibit these processes. ***Nowhere in the prior art or in Dudek et al, is there any direct and unambiguous disclosure that cyclopamine or a derivative thereof may be used for inducing differentiation and/or apoptosis of said tumor cells.***

The cellular proliferation, differentiation and apoptosis processes play crucial roles in the generation and functioning of multicellular organisms. Accordingly, multiple and complex mechanisms exist for their regulation.

It is well known in scientific literature that inhibition of cellular proliferation cannot be regarded as an anticipator of cellular differentiation or apoptosis.

In multicellular organisms, the requirement for generation of different cellular phenotypes by use of the same genomic information necessitates utilization of different sets of signals and complex regulatory mechanisms for cellular differentiation. A priori, inhibition of cellular proliferation may have no effect on induction of differentiation or it may be stimulatory or else, it may inhibit induction of differentiation. These possibilities have been investigated in numerous studies and it is firmly established that the results depend on the cell types used as well as the agents/stimuli used.

It is well-known in the art that ***molecules designed to inhibit cellular proliferation specifically*** (e.g., by specific inhibition of the replication of nuclear DNA) ***do not commonly induce tumor cell differentiation.*** In many tumor cell populations, proliferation can indeed be inhibited effectively by many agents without inducing differentiation. It is again well-known that treatment of malignant cells with known inducers of differentiation can result in induced cells that continue to proliferate for several cell cycles.

Furthermore, it is well-established in scientific literature that ***differentiation of many normal cell types requires proliferation for one or more cell cycles.*** Similarly, induction of tumor cell differentiation by known inducers of differentiation has also been found, in numerous well-studied examples, to be absolutely dependent on cellular proliferation for one or more cycles. The replication-associated remodeling of chromatin appears to be an important reason behind this requirement of proliferation for differentiation.

There are well-known situations, accordingly, where stimulation of proliferation also stimulates differentiation. For example, hydrocortisone is well-known to be a stimulator of the proliferation of squamous cell carcinoma (SCC) cells, and at the same time, it is a stimulator of the differentiation of SCC cells. Consequently, inhibition of cellular proliferation cannot be considered as an anticipator of cellular differentiation. The outcome clearly depends on the cell types as well as on the nature of the agents used. A further relevant observation made in the scientific literature is that cessation of cellular proliferation following completion of a differentiation program along a cell lineage is commonly a consequence of cellular differentiation rather than being a cause.

Cyclopamine, which binds to the *Smoothened* protein, is known to be a specific inhibitor of *Hh/Smo* signalling. *Dudek et al* and other prior art documents describe it as an inhibitor of cellular proliferation. Since the *Hedgehog* proteins were discovered originally as inducers of cellular differentiation during development (a property well confirmed since), ***inhibition of Hh/Smo signaling*** (by cyclopamine or otherwise) ***would ordinarily be expected to inhibit cellular differentiation.*** The prior art, describing inhibition of differentiation of a number of cell types as a result of exposure to cyclopamine had confirmed this anticipation. Moreover, the influence of inhibiting cellular proliferation by cyclopamine had been studied in prior art specifically with regard to its effects on cellular differentiation (Detmer K et al., *Blood Cells Molecules and Diseases* 2000;26:360-372). Inhibition of proliferation by cyclopamine was found to inhibit cellular differentiation in a well-established model system (differentiation of bone marrow cells).

The cited prior art document (*Dudek et al*) does not present any data on the influence of the inhibition of *Hh/Smo* signaling on cellular differentiation. On the other hand, references are made to the published studies in literature reporting inhibition of differentiation by the inhibition of *Hh/Smo* signaling. Besides these, *Dudek et al* contains directly conflicting disclosures concerning the influence of the inhibition of *Hh/Smo* signaling on differentiation (e.g., of epithelial cells) and is replete with ambiguous/unclear statements concerning the influence of said inhibition on differentiation (cell differentiation is disclosed to be "altered", "affected", "controlled", "regulated", "managed", "modulated" or "manipulated" by a subject compound; see below). Likewise, inspection of whole prior art shows that no clear or enabling disclosure existed therein that inhibition of *Hh/Smo* signaling could be used to induce differentiation of tumor cells.

Similar to the situation with cellular differentiation, extensive studies in scientific literature indicate that ***inhibition of tumor cell proliferation cannot be equated with the induction of apoptosis*** and that selection of specific agents, means of application as well as the nature of treated cells are all important in determining whether or not apoptosis would be induced. In fact, with some inhibitors of proliferation and tumor cell types, it is known that inhibition of proliferation renders tumor cells resistant to apoptosis. Accordingly, with some conventional chemotherapeutics, prior or concomitant administration of proliferation stimulators has been well established to provide greater tumor cell killing. In normal cells, too, nature of the cells and means of proliferation inhibition are known to be important in determining whether or not apoptosis would be induced and, again, inhibition of proliferation may render cells resistant to apoptosis. Furthermore, most illustratively, transgenic mice overexpressing the growth factor GM-CSF in epidermis have been found to show both increased proliferation and increased apoptosis of epidermal cells in vivo.

Influence of cyclopamine and other inhibitors of *Hh/Smo* signaling on cell viability/apoptosis had also been evaluated in prior art with transformed cells. *Beachy PA* (WO 01 27135 A2) had described experiments in which transformed cells were treated with varying concentrations of cyclopamine or a derivative thereof and disclosed that "*cell death ... was not observed even at the highest doses of compounds used*" (page 13, lines 31-32). Similarly, *Taipale et al* (Taipale J et al., *Nature* 2000;406:1005-1009) had described experiments where cyclopamine and a derivative thereof were indicated not to have caused death of transformed cells at concentrations causing complete inhibition of cellular proliferation (page 1007, Figure 3 legend, left column, lines 2-5). In addition, even though microscopic examinations of transplanted tumors were performed by both *Beachy PA* (WO 01 27135 A2, pages 132-133) and *Dudek et al* (Column 63 line 51 to Column 64 line 22) following treatment in vivo with cyclopamine or a derivative thereof, induction of tumor cell apoptosis was not disclosed in either study. The prior art is lacking, in its entirety, a clear or enabling disclosure that cyclopamine or another specific inhibitor of *Hh/Smo* signaling can be used to induce tumor cell apoptosis in vitro or in vivo.

It is thus clear that ***inductions of tumor cell differentiation and apoptosis by use of cyclopamine or a derivative thereof cannot be considered to be inherent or automatic technical effects.*** The prior art had in fact employed cyclopamine or a derivative thereof on transformed cells *in vitro* and on tumor cells *in vivo* and concluded that they do not cause cell killing, even at the highest concentrations causing complete inhibition of proliferation. This is simply the opposite of what the present application discloses. Thus there is no anticipation of the method claims 1-12 and 25-27.

As for obviousness, *Dudek et al* has no unambiguous disclosure or documentation of tumor cell differentiation following exposure to cyclopamine or a derivative thereof. Rather, the weight of evidence in prior art pointed in the opposite direction. On the other hand, studies published since the filing of this application have confirmed that responses of cells to cyclopamine in terms of both cellular differentiation and apoptosis depend, indeed, on the cell types, the environment in which the cells reside (e.g., the environment in vivo or ordinary in vitro culture conditions) as well as on the means, extent and duration of the inhibition *Hh/Smo* signaling. For example, *Hh/Smo* signaling in skin is stimulated in proportion to the β-catenin signaling and caused sebocyte differentiation at intermediate levels (Celso CL et al., *Development* 2004;131:1787-1799). However, higher levels and duration of signaling caused rather inhibition of the sebocyte differentiation (ibid). Moreover, while continuous high level *Hh/Smo* signaling in the skin of transgenic mice resulted in hair follicle type tumors, inhibition of signaling by ΔNLef1 led to the formation of sebaceous tumors (ibid). We have also disclosed that inductions of tumor cell differentiation and apoptosis are sensitive to how long and how complete an inhibition of the *Hh/Smo* signaling is caused (see e.g. the examples shown in Figures 5A-F and 7A-F and the related descriptions in the specification). Similarly, both the duration of

Hh/Smo signaling and the level of signaling have now been shown to be critical in determining the subtypes of neurons that are induced to differentiate from precursors of neurons (Stamataki D et al., Genes & Development 2005; 19: 626-641).

Dudek et al in particular, and the prior art in general, teach inhibition of the proliferation of the cells having *Hh/Smo* signaling activity by contacting said cells with an inhibitor of said signaling, such as cyclopamine. The cells may be transformed cells or tumor cells and said contacting may be performed in vitro or in vivo, according to the prior art. However, nowhere in *Dudek et al* is there an unambiguous or enabling disclosure is found that cyclopamine or a derivative thereof can be used to induce differentiation and/or apoptosis of tumor cells. It is also well established in art that inhibition of cellular proliferation cannot be considered to anticipate inductions of cellular differentiation or apoptosis. The results depend very much on the particular cell types, their environment as well as on the means of inhibition of proliferation and, indeed, there are well-known situations where inhibition of proliferation is inhibitory to the inductions of differentiation or apoptosis.

Furthermore, with respect to the induction of apoptosis, tumors are known to undergo decrease of size *in vivo* also for reasons other than the induction of apoptosis, for example due to causation of necrosis. However, removal of tumor cells by apoptosis is preferred for several reasons, including the severe inflammatory reaction that follows necrosis but not apoptosis.

Still further, rapid shrinking and disappearance of tumors, owing to the efficiently and selectively induced massive apoptotic removal of tumor cells, particularly benefits those patients in whom slowing or cessation of tumor growth would not be sufficient because of, *e.g.*, anatomical position resulting in exertion of pressure on vital structures.

Induction of the differentiation and/or apoptosis of tumor cells *in vivo* with the efficiency providing complete disappearance of tumors and with the selectivity sparing normal cells were not only novel and met a long-felt need but also highly unexpected in view of prior art. The inhibition of Hh/Smo signaling by cyclopamine or a derivative thereof was disclosed explicitly in prior art not to cause any cell death in transformed cells, even at the highest concentrations causing complete inhibition of proliferation. Also, Hedgehog proteins were well-known as inducers of cellular differentiation. Thus, in prior art the skilled person was taught away from the present invention; he/she would not even contemplate to find conditions where the technical effects described in this application would be achieved. This is made even more evident by considering the following prior art disclosures teaching away from the invention.

A. A well-known problem of the conventional cancer chemotherapeutics and of radiation therapy is the causation of damage to the normal stem cells. Yet these cells are found to be dependent on Hh/Smo signaling for their maintenance *in vivo* (Zhang Y et al., Nature 2001;410:599-604).

B. Cyclopamine is a teratogen (Keeler RF, Phytochemistry 1969;8:223-225).

C. Administration of cyclopamine to tumor-bearing mice made the animals sick to such an extent that one in three had to be killed (Dudek et al, Column 63 lines 61-63).

D. In prior art, induction of tumor cell apoptosis in vivo by non-genotoxic means and in a highly efficient and selective manner so as to obtain disappearance of tumors while preserving normal cells was viewed as unattainable on theoretical grounds (Hannun YA, Blood 1997;89:1845-1847).

At least for the reasons given above the subject matter of claims 1-12 and 25-27 is neither anticipated nor obvious from *Dudek et al.* Withdrawal of the rejections of claims 1-12 is respectfully requested.

Claims 13-21 and 28

In the Office Action Claim 13 has been considered to recite an intended use that does not a limitation on the claimed subject matter. Also, the Office Action indicates that Takano et al is considered anticipating of claim 13, although a formal rejection has not been given.

Takano et al has not been identified sufficiently for applicant to understand what document is being cited. If the Examiner wishes to rely on this document it should be identified sufficiently to applicant to locate a copy.

Claim 13 has been amended to remove the intended use recitation and to add the recitation of a mediciment "*in a dosage form effective for inducing in vivo the cellular differentiation and/or apoptosis of tumor cells that use the hedgehog/smoothened signalling for proliferation and for prevention of apoptosis and/or for prevention of differentiation*" (emphasis added). From the foregoing discussion of *Dudek et al,* and of the prior art in general, is clear that cyclopamine or a derivative thereof has not previously been provided in such a dosage form and that such a dosage form would not have been considered obvious from the prior art use of such compounds. Accordingly, withdrawal of the rejections of claims 13-21 is respectfully requested.

New claim 28 is directed to alternates to cyclopamine that are taught in the specification and are patentable over Dudek et al.

Claim 23 and 27

Claim 23 has been amended to recite a method for causing decreased pigmentation of nevi or hyperpigmented skin areas. New method claim 27 is likewise directed to a method for causing decreased pigmentation of nevi on hyperpigmented skin areas. Amended claim 23 and new claim 27 are not anticipated by *Dudek et al.*

Dudek et al disclose that Forskolin causes decreased pigmentation of hair and *Shh* causes increased pigmentation of hair (Col.67 lines 48-59). As elevations of intracellular cAMP are well-known to affect and to be caused by various intracellular events and signaling pathways that are distinct from and unrelated to the *Hh(Smo* signaling, the elevation of intracellular cAMP by Forskolin cannot be regarded as specific to the inhibition of *Hh/Smo* signaling. Column 67 lines 7-21 of *Dudek et al* refers in fact to the elevation of intracellular cAMP by many other signaling pathways that are distinct from *Hh/Smo* signaling. While *Shh* is disclosed to cause increased pigmentation of hair, there is no disclosure of its influence on the pigmentation of skin. Also, there is no disclosure in the prior art or in *Dudek et al* that cyclopamine or a derivative thereof can be used to decrease the pigmentation of skin or of nevi. Furthermore, a person skilled in the art knows well that the pigmentation of skin and the pigmentation of hair are regulated separately and that the two are divorced from each other in many physiological and pathological situations (consider e.g., the graying of hair in a dark skinned person). Therefore *Dudek et al* does not teach or suggest a decrease in skin pigmentation. Accordingly, withdrawal of the rejection of claim 23 is respectfully requested.

Double Patenting

Claims 13-15, 17 19, 20 and 22-24 have been provisionally rejected under 35 USC §101 as claiming the same invention as claims 12-17 of copending application 10/682,662. To remove the basis for this rejection an amendment of application 10/682,662 to cancel claims 12-17 is being filed concurrently herewith. The amendment removes the ground for rejection and so withdrawal of this provisional rejection is respectfully requested.

Conclusion

In view of the foregoing amendments and remarks the pending claims are seen to recite subject matter that is enabled, definite, patentable over the cited art and not in double-patenting conflict with applicant's copending application. Accordingly the application is believed to be in condition for allowance. Early and favorable action is requested.

Respectfully submitted,

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